

Differences in Breast Cancer Subtypes

A short analysis of “Loss of Wwox drives metastasis in triple-negative breast cancer by JAK2/STAT3 axis” by Chang et. al

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Background and motivation

- Around 1 in 8 women will develop breast cancer during their lifetime, making it the second most common cancer in women and second leading cause of cancer death in women [6].
- However, not all breast cancers are “created” equal, with some more aggressive sub-types being associated with worse prognosis [2].
- Thus, with advancements in bioinformatics and molecular profiling, the genetic differences between sub-types have become a huge interest in recent years for prognostic and therapeutic applications [3].

Molecular Sub-types

- **Luminal A** - BC that is hormone-receptor positive, HER2 negative, and has low levels of protein Ki-67
- **Luminal B** - BC that is hormone-receptor positive, either HER2 negative or positive, with high levels of Ki-67
- **Triple-negative/Basal-like*** - BC that is hormone receptor negative and HER2 negative
 - ▶ Harder to treat and more aggressive
 - ▶ More common in younger women, Black/African American, or BRCA mutation
- **HER2-enriched** - BC that is hormone receptor negative and HER2 positive.

WW Domain Containing Oxidoreductase

- Wwox is a protein coding gene/enzyme that acts as a transcription regulator to mediate action of cytokines, interferon, and growth hormone [1].
- There is evidence that this protein may also function as a tumor suppressor and may work with P53/TP53 to control stress induced cell death and tumor necrosis factor-mediated cell death.

Janus kinase and signal transducer and activator of transcription

- The JAK/STAT pathway is a signaling pathway which causes a chain of reactions between proteins in a cell.
- It is involved with such processes as immunity, cell division, cell death, and tumor formation [5].
- Deregulation of the pathway (either over/under) has been linked to many diseases, including immune system disorders and cancers.

The paper

- This study followed the paper “Loss of Wwox drives metastasis in triple-negative breast cancer by JAK2/STAT3 axis” by Change et. al [3].
- In one section of their paper, the researchers used gene expression analysis and pathway analysis to compare luminal to basal BC types.
- Their conclusions included
 - ▶ Wwox is down-regulated in basal BC compared to luminal breast cancer.
 - ▶ The JAK/STAT pathway was one of the most up-regulated pathways between basal-like and Luminal type BC.
 - ▶ The lower expression of Wwox was associated with activation of STAT3 in basal-like BC while over expression of Wwox inhibited proliferation and metastasis.

Objective

- The purpose of this analysis was to perform gene expression and pathway analysis on the expression data provided by the aforementioned study to compare if we received similar results, particularly for the Wwox gene and JAK/STAT pathway.

The Data

- The gene expression data was made available on the GEO profiles database under the accession code GSE110810.
- There were 6 libraries in total consisting of BC cells lines purchased from American Type Culture Collection.

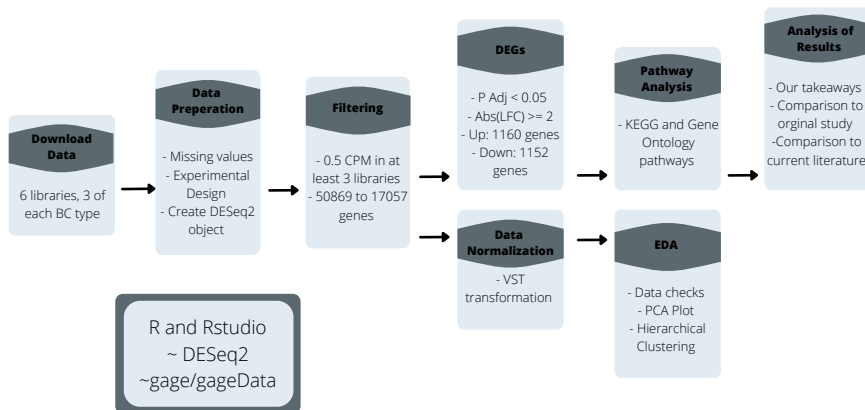
- ▶ **Luminal**

- ★ MCF-7
- ★ T47D
- ★ BT-474

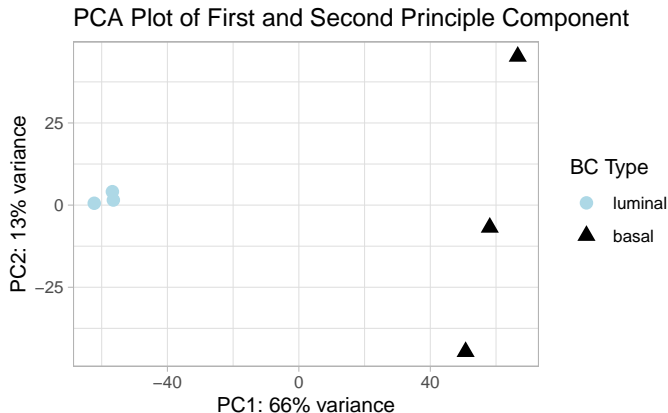
- ▶ **Basal**

- ★ SUM-159
- ★ HBL-100*
- ★ BT-549

Workflow

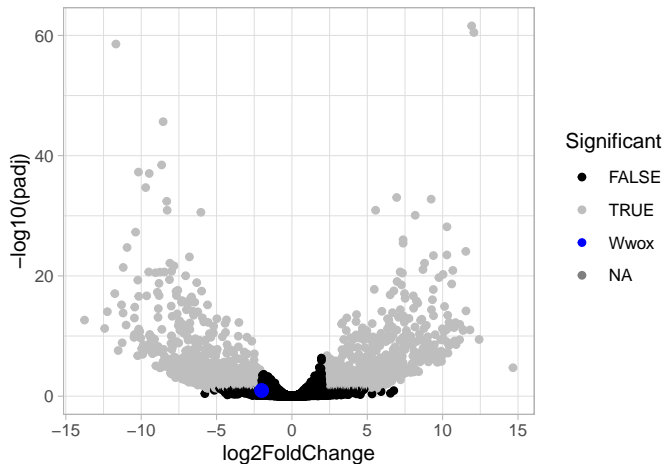


Exploratory Data Analysis - PCA



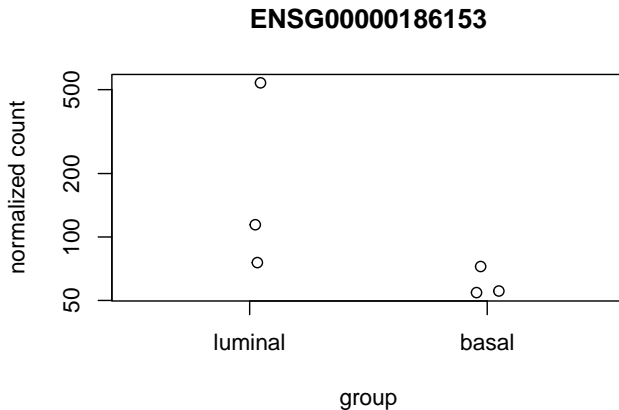
There is a clear difference between luminal and basal breast cancers. The first PC explains 66 percent of the data and separates the two sub-types.

Differently Expressed Genes



Wwox was down-regulated but not significant.

Normalized counts of Wwox



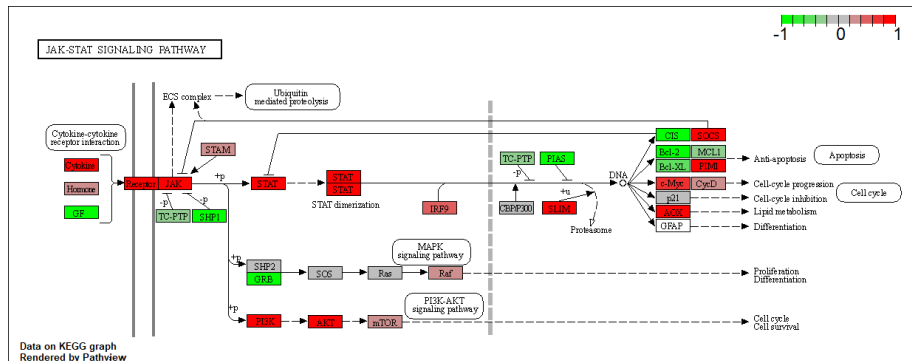
Two of the luminal cell lines have higher normalized counts of the Wwox gene than the basal cell lines.

Pathway Analysis - KEGG

Table: Top five KEGG pathways by stat mean

Process	Direction	Mean	P-Value
Focal adhesion	Greater	5.11	$2.76e^{-7}$
ECM-receptor interaction	Greater	4.56	$6.81e^{-06}$
Complement and coagulation cascades	Greater	3.46	$5.3e^{-04}$
Protein digestion and absorption	Greater	3.119	$2.75e^{-03}$
Jak-STAT signaling pathway	Greater	2.53	$6.03e^{-03}$

JAK/STAT Pathway



Several of the genes in this pathway are up-regulated (red).

Discussion

- The BC sub-types have a lot of differences in gene expression.
- Wwox was not found to be significant in our analysis.
 - ▶ LFC does suggest it was down-regulated in basal BC.
 - ▶ Relatively low level of counts compared to genes such as SPARC.
 - ▶ The gene is a transcription regulator.
 - ▶ Thus we found that additional research is needed on this gene.
- JAK/STAT pathway was significant.
 - ▶ The signaling pathway was up-regulated in basal BC.

Limitations

- The use of cell lines may not provide the full biological picture.
- The choice of HBL-100 was an interesting choice as some sources suggest not to use it [4].
- Basal-like and triple negative cancers should not be used interchangeably [2].

Going Further

- Perform gene expression analysis on knocked down Wwox gene in luminal BC and over-expressed Wwox in basal BC.
- Complete gene expression analysis on direct tissues samples from a larger population of women.

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